

Hypoglycemia and Hyperinsulinemia in Rodent Models of Severe Malaria Infection

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Severe hypoglycemia developed during nonlethal *Plasmodium chabaudi* and lethal *P. yoelii* blood stage malaria infection in mice, always in association with hyperinsulinemia. Supernatants of lethal *P. yoelii* incubated overnight induced hypoglycemia and hyperinsulinemia in normal mice. In murine malaria, hypoglycemia may be largely secondary to increased insulin secretion.

Hypoglycemia (blood glucose of <4 mmol/liter) is recognized as a serious complication of *Plasmodium falciparum* malaria (20), but the triggering mechanism is unclear. In patients treated with quinine, it is usually attributed to the hyperinsulinemic action of quinine (11, 19), though cases in which hypoglycemia (10, 17) and hyperinsulinemia (10) preceded treatment have been reported. We recently showed, using the murine malaria parasite *P. yoelii*, that a supernatant of blood stage parasites incubated overnight induced hypoglycemia when injected into normal mice and that this could be prevented by prior immunization with supernatant or with phosphatidylinositol (15); we proposed that the “toxic antigens” responsible were phospholipids similar to those we had already shown to induce tumor necrosis factor (TNF) (1). It was subsequently proposed by another laboratory that a glycosylphosphatidylinositol molecule derived from *P. falciparum* had similar activities (13). We also found, by measuring lipogenesis and lipolysis in adipocytes in vitro, that these toxic antigens appeared to synergize with low levels of insulin rather than acting on their own (16). The way in which these parasite-derived molecules induce hypoglycemia is not established, but in view of the hyperinsulinemia reported in hypoglycemic malaria patients treated with quinine (11, 19), we thought it worth investigating the role of insulin in the production of hypoglycemia in our experimental animal models of malaria.

Mice, parasites, and assays. We used 8- to 12-week-old (C57BL \times BALB/c)F1 mice bred in our department from parent strains obtained from the National Institute for Medical Research, London, England. Lipopolysaccharide-unresponsive C3H/HeJ mice were obtained from Harlan Olac Ltd. (Bicester, England). Outbred CD1 mice were obtained from Charles River Ltd. (Kent, England). Mice were kept on a standard 12-h light–12-h darkness cycle and pellet diet (RMI; SDS, Essex, England). They had free access to food and water throughout the experiment. The nonlethal strain of *P. yoelii*, 17X, was donated by the London School of Hygiene and Tropical Medicine. The lethal *P. yoelii* YM variant was obtained from A. Holder, then at Wellcome Biotech. The *P. chabaudi* strain used was the nonlethal AS strain, obtained from K. N. Brown, NIMR. All parasites were maintained by blood passage, and infections were initiated intravenously with 10^4 parasitized erythrocytes. Parasitemia was scored on Giemsa-stained tail

blood films. Parasite supernatants were prepared with the YM lethal variant of *P. yoelii*, following overnight culture of parasitized blood in phosphate-buffered saline (pH 7.4) on a roller at 37°C, as described previously (1). For the determination of immunoreactive insulin (IRI), blood was collected from the trunk in heparinized Eppendorf tubes following decapitation of the mice. Plasma was separated by centrifugation and frozen at -20°C . IRI concentrations were determined in 50- μl volumes by the double-antibody radioimmunoassay technique (7), using antibody and ^{125}I -labelled insulin (kit supplied by ICN Biomedicals, Irvine, Calif.) and a crystalline rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark), widely used for mouse work (14). All samples were assayed in duplicate. Glucose concentrations were determined enzymatically on 10- μl volumes of tail blood, collected between 10 a.m. and midday, using Glucostix and an Ames Glucometer (Mile Ltd., Stoke Poges, England). Measurements from more than 50 normal mice of both sexes gave a value of 7.0 ± 0.5 mmol/liter. Blood glucose levels millimoles per liter and plasma IRI values (nanograms per milliliter) are expressed as means \pm standard errors of the mean (SEM). Statistical significance was assessed by using analysis of variance (ANOVA) or, when appropriate, Student's *t* test for paired observations.

Hypoglycemia and hyperinsulinemia. Blood glucose was measured daily during infection with nonlethal *P. yoelii* (17X), lethal *P. yoelii* (YM), or *P. chabaudi* (Fig. 1a, b, and c). Nonlethal *P. yoelii* did not induce hypoglycemia. With *P. yoelii* YM, hypoglycemia occurred 1 to 2 days before death, when the parasitemia was around 50%. With *P. chabaudi*, the mice became severely hypoglycemic at days 10 to 12, at the time of highest parasitemia (60%) and crisis, but recovered shortly thereafter. Hypoglycemia was identical in C3H/HeJ mice (not shown), suggesting that endotoxin was not a factor.

Before infection, mice had 2.5 ± 0.2 ng of insulin per ml in their blood, which is within the expected range (18). During *P. yoelii* YM infection, plasma IRI levels rose with the parasitemia, to reach the remarkably high level of 180 ng/ml on the day before death (Fig. 2a). During *P. chabaudi* infection, IRI levels rose to 90 ng/ml 11 days after infection, at a time when parasitemia was about 60%, and fell as the mice recovered (Fig. 2b). Thus, in both cases, blood glucose and insulin levels were inversely correlated.

As reported previously (15), injection of parasite supernatants induced a significant fall of blood glucose in normal mice, reaching a minimum at 4 to 6 h ($P < 0.0001$; Fig. 3a). Supernatants of normal erythrocytes were weakly active or negative. Four hours after injection of parasite supernatants,

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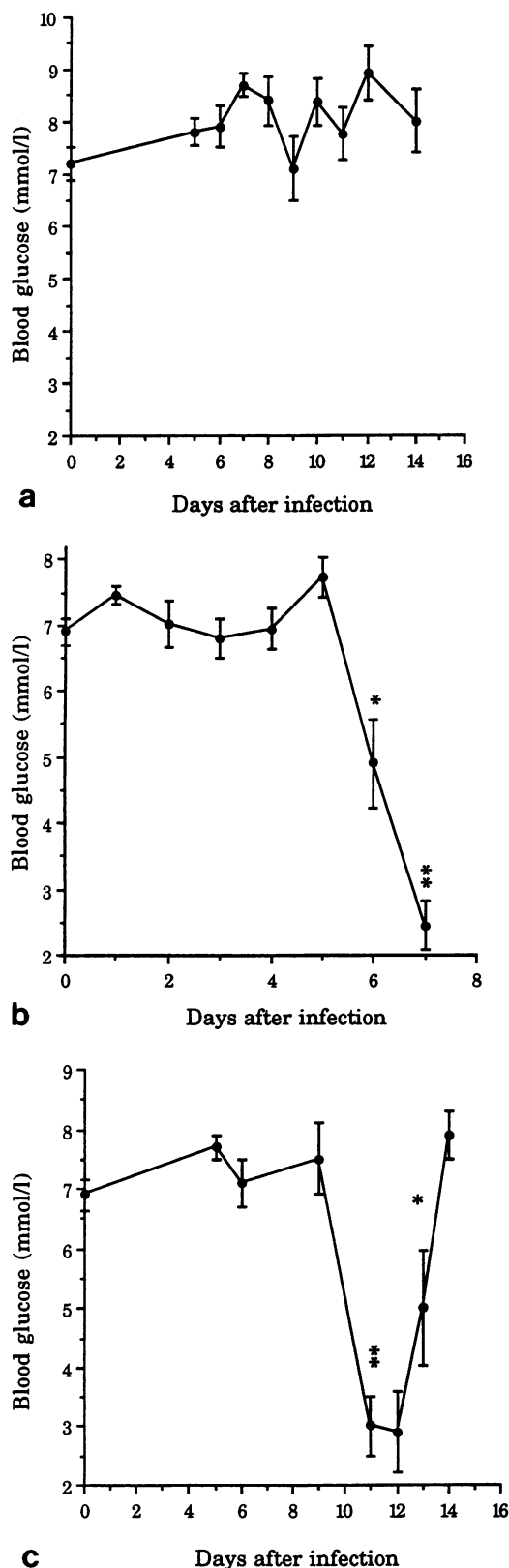


FIG. 1. Blood glucose levels during infection of (C57BL \times BALB/c)F1 mice with nonlethal *P. yoelii* 17X (a), lethal *P. yoelii* YM (b) and (c) *P. chabaudi* AS (c). Values are means \pm SEM (six to nine mice per experiment). ANOVA shows a significant effect of lethal *P. yoelii*

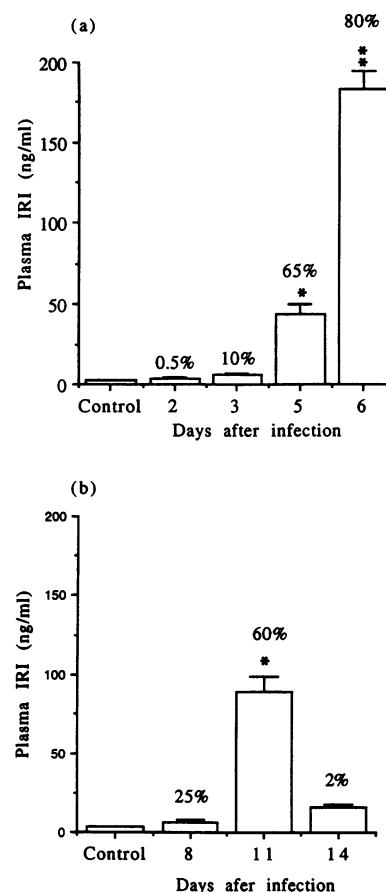


FIG. 2. Plasma IRI levels in groups of 8 to 10 mice infected with lethal *P. yoelii* (a) or *P. chabaudi* (b). Each column represents the mean \pm SEM. ANOVA shows a significant effect of the infection compared with uninfected (control) values: * $P < 0.05$ and ** $P < 0.0001$. The mean percent parasitemia is given above each column.

there was a significant increase in plasma insulin levels from 2.2 ± 0.2 to 5.2 ± 0.7 ng/ml ($P < 0.01$; Fig. 3b). The supernatants themselves gave readings of 0 to 0.2 ng/ml, so the amount of insulin contained in 0.5 ml (the injected dose) could not account for the blood levels detected.

The exact mechanism(s) of hypoglycemia in malaria remains controversial, but insulin appears to play a role in murine models, according to the present study. It was noteworthy that hypoglycemia and hyperinsulinemia were only detected when the parasitemia exceeded about 50%, which may explain why they were not seen in the nonlethal *P. yoelii* infection, when parasitemia rose to only about 20%. Such levels are rare in human malaria, although if parasites sequester in the pancreas, as they do in the brain, the local concentration could be relatively high.

It has been suggested that high levels of circulating TNF are responsible for much of the pathology of malaria (6). Infusion of TNF in animals has been reported to increase glucose uptake but also to cause a decrease in serum insulin (3). Moreover, injection of a neutralizing monoclonal antibody

infection compared with uninfected (day 0) values, * $P < 0.05$ and ** $P < 0.0001$, and a significant effect of *P. chabaudi* infection, * $P < 0.05$ and ** $P < 0.0001$. No significant effect is seen with nonlethal *P. yoelii*.

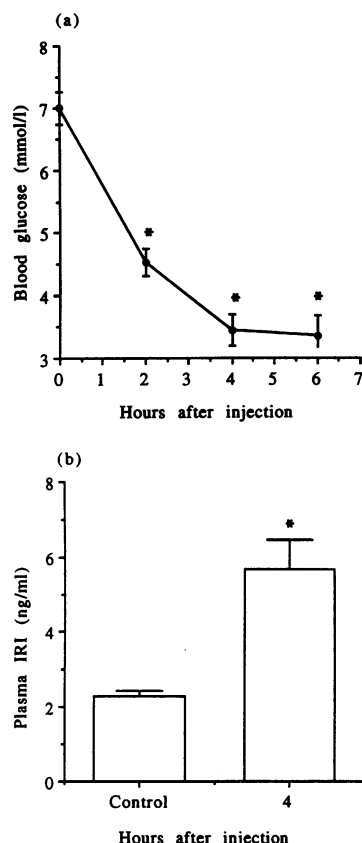


FIG. 3. Blood glucose (a) and plasma IRI (b) levels in normal mice after injection of 0.5 ml of lethal *P. yoelii* parasite supernatant. The results are pooled from three experiments with different supernatants (three mice per experiment). Values are means \pm SEM. ANOVA shows a significant effect of the supernatant compared with normal (time zero; control) values: * $P < 0.0001$.

against murine TNF failed to attenuate hypoglycemia induced by our malaria supernatants (15), so it seems unlikely that TNF plays a major role in the marked hyperinsulinemia we observed. We are currently investigating the possibility that malaria-derived molecules may directly stimulate insulin secretion from pancreatic islets, as appears to be the case with a preparation derived from *Bordetella pertussis* which increases insulin secretion and lowers blood glucose in normal but not pancreatectomized dogs (22); a similar effect is seen in *B. pertussis*-infected mice (14) and in rats injected with *Salmonella* endotoxin (21) or with quinine (8). The higher levels of insulin associated with hypoglycemia in the infected mice than in the supernatant-injected mice (Fig. 2a versus 3b) could be explained by the development of insulin resistance during infection (2), possibly due to TNF (4), but it is possible that factors other than insulin also contribute to hypoglycemia.

There are many differences between murine and human malaria, but it might be of value to measure plasma insulin in a larger number of patients with severe malaria, particularly in those with high parasitemia. If there is indeed a significant subgroup in which hyperinsulinemia is a feature, therapy might be directed at this. Indeed, in one study, somatostatin analog (SMS 201-995) was successfully used to control hyperinsulinemia in quinine-treated patients (11). Treatment along these lines might be more logical than injection of glucose, which is likely to further stimulate insulin secretion. Hypoglycemia with

normal plasma insulin is more likely to be due to depletion of liver glycogen or glucose starvation, and here, of course, glucose therapy would be indicated.

Hypoglycemia has been reported previously in mice (12) and rats (9) infected with *P. berghei* and in rhesus monkeys with *P. knowlesi* (5) and *P. coatneyi* infection (2). However, ours appears to be the first report of self-limiting hypoglycemia during infection with nonlethal *P. chabaudi* and the first to show the expected correlation with insulin levels, namely, hyperinsulinemia at the time of hypoglycemia. This may therefore be a good model in which to study the effects of malaria on glucose homeostasis.

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